Production of Haploid Plants

Haploid plants are characterized by possessing only **a single set of chromosomes.** Haploid plants are of great significance for the production of **homozygous plants** and for the improvement of plants in plant breeding programs.

History:

1. The existence of haploids was discovered by **Bergner** in ***Datura stramonium.***
2. The Indian scientists **Guha and Maheswari** (1964) reported the direct development of haploid embryos and plantlets from microspores of ***Datura innoxa*** by the anther culture.
3. Later, **Bourgin and Hitsch** (1967) obtained haploid plants from ***Nicotiana tabacum.***

Steps involved in anther culture:

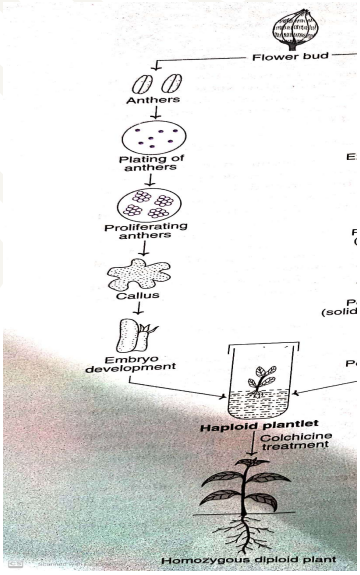
Step 1. The selected flower buds of young plants are surface sterilized and anthers removed along with their filaments.

Step 2. The anther are excised (cut) under aseptic conditions and crush in 1% acetocarmine to test the stage of pollen development.

Step 3. If they are at the correct stage, each anther is gently separated from the filament and the intact anthers are inoculated on a nutrient medium.

Step 4. the anther culture maintained in alternating periods of light (12-18hr) and darkness (6-12 hrs) at 28◦C.

Step 5. As the anthers proliferate, they produce callus which later forms an embryo and then a haploid plant.



Steps involved in pollen culture:

Step 1. Haploids plant are produced from immature pollen or microspores.

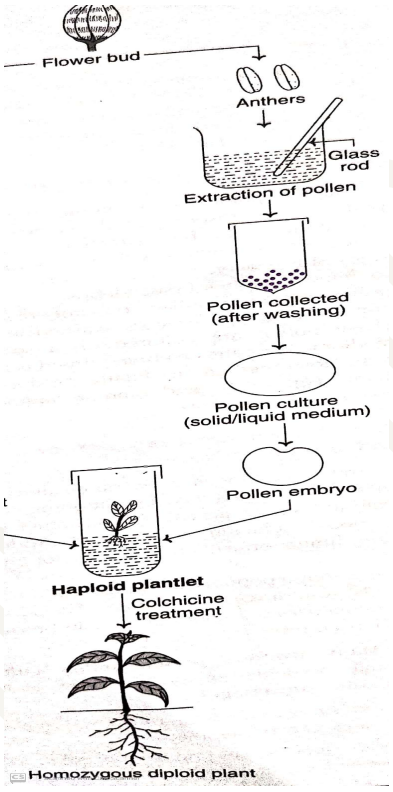
Step 2. The pollen are extracted by pressing and squeezing the anthers with a glass rod against the sides of a beaker.

Step 3. The pollen suspension is filtered to remove the anther tissue debris.

Step 4. Viable and large pollen (smaller pollen do not regenerate) filtered again , washed and collected.

Step 5. These pollen are cultured on a liquid or solid medium.

Step 6. After callus or embryo formation, they are transferred into a suitable medium.



Development of Haploid plant:

The cultured pollen or microspores mainly follow four distinct pathways to develop a haploid plants in *in vitro.*

Pathway I : The uninucleate microspore undergoes equal division to form **two daughter of equal size**. e.g., ***Datura innoxa***.

Pathway II: The microspore divides unequally to form large **vegetative cell** and smaller **generative cell**. Later, the **vegetative cell** will form callus. e.g., ***Nicotiana tabacum, Capsicum annuum***.

Pathway III: Here, the microspore undergoes unequal division and form vegetative cell and generative cell. Later, The **generative cell** will form embryo/ callus. e.g., ***Hyoscyamus niger***.

Pathway IV: The microspore unequally divided into vegetative and generative cell. Later, both vegetative and generative cell will form callus / embryo . So that haploid plant will developed. E.g., ***Datura metel, Atropa belladonna***.

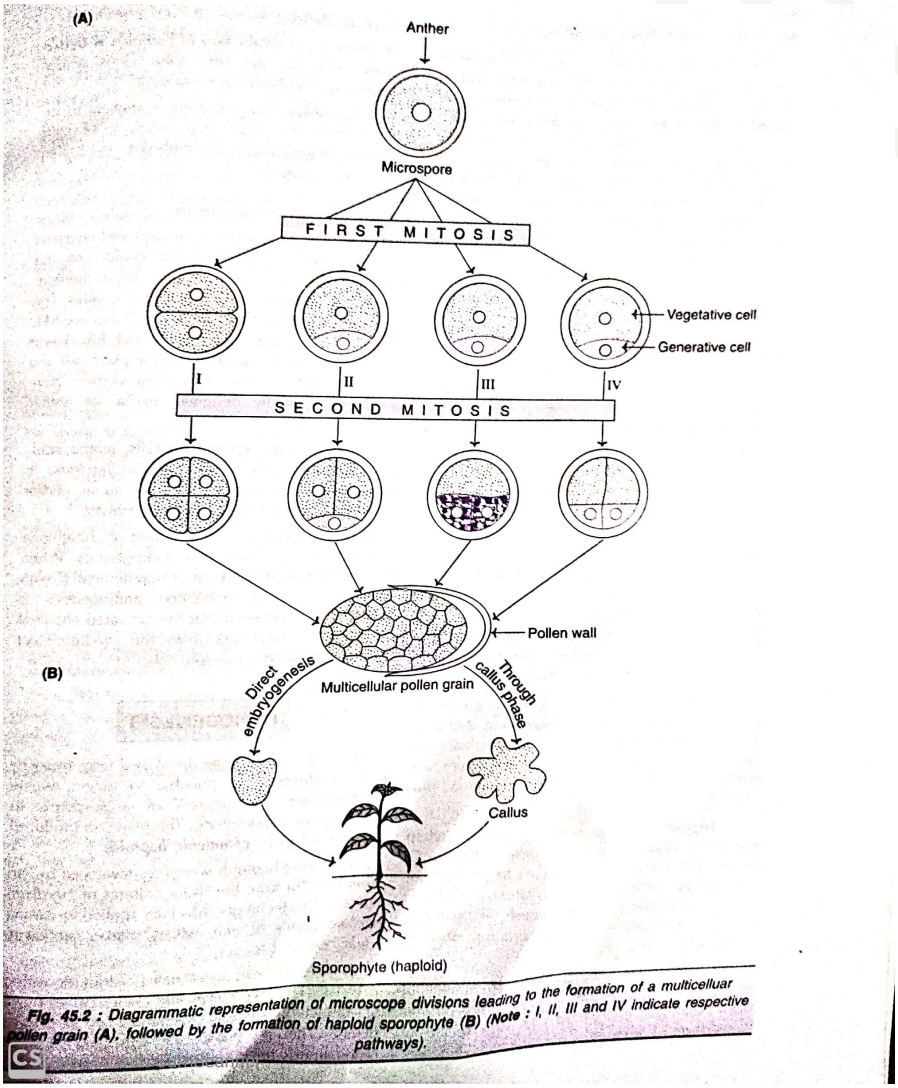


Fig. 45.2 : Diagrammatic representation of microscope divisions leading to the formation of a multicelluar pollen grain (A), followed by the formation of haploid sporophyte (B) (Note : I, II, III and IV indicate respective pathways).

Factors affecting the pollen culture/ anther culture:

1. **Genotype of the donor plant**: the plant having highly responsive genotypes.
2. **Stage of microspore or pollen**: Tetrad or binucleate stage are more responsive.
3. **Physical status of the donor plant**: should be healthy, flowering in season etc.
4. **Pretreatment of anther**: Chemical treatment should be appropriate
5. **Effect of light**: Light and dark duration should be appropriate.
6. **Effect of culture medium**: The culture medium should be very appropriate both quantitatively and qualitatively.

Applications of Haploid plants

1. Development of homozygous plants or lines
2. Generation of exclusive male plants
3. Induction of mutations
4. Production of disease resistance plants
5. Production of insect resistance plants
6. Production of salt tolerance plants
7. Cytogenetic research
8. Induction of genetic variability
9. Double haploid in genome mapping
10. Evolutionary study